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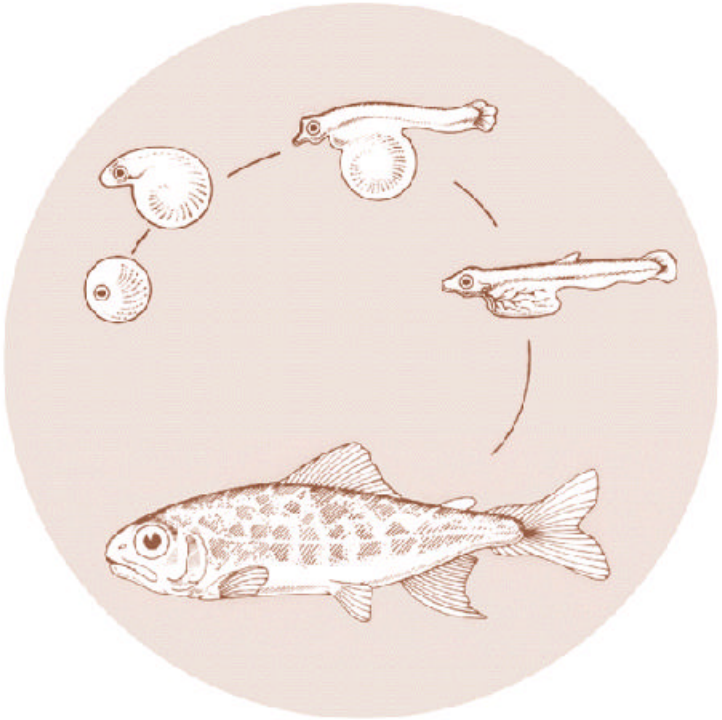
EPIDEMIOLOGY AND CONTROL OF INFECTIOUS DISEASES OF SALMONIDS IN THE COLUMBIA RIVER BASIN

Annual Report 1983



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Epidemiology and Control of Infectious Diseases of Salmonids in the Columbia River Basin

Annual Report FY 1983

by

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ABSTRACT

The Department of Microbiology at Oregon State University with funding from the Bonneville Power Administration conducted a study relating to the epidemiology and control of three fish diseases of salmonids in the Columbia River Basin. These three diseases were ceratomyxosis which is caused by the myxosporidan parasite Ceratomyxa shasta, bacterial kidney disease, the etiological agent of which is Renibacterium salmoninarum, and infectious hematopoietic necrosis, which is caused by a rhabdovirus. Each of these diseases is highly destructive and difficult or impossible to treat with antimicrobial agents.

The presence of ceratomyxosis in rainbow trout exposed at McNary and Little Goose Dams extends the range of this disease about 200 miles further up the Columbia River and into the Snake River drainage. Wallowa steelhead trout were less resistant to this disease than other upriver stocks tested. Juvenile salmonids entering the Columbia River estuary were collected periodically between May to September, 1983. Nine percent of the beach seined chinook salmon and 5, 11 and 12 percent, respectively, of the purse seined coho and chinook salmon and steelhead trout were infected with Ceratomyxa shasta. Experiments indicated ceratomyxosis progresses in salt water at the same rate as in fresh water once the fish have become infected. This data, summarized, indicates a longer exposure to infective stages of C. shasta than previously identified and that approximately 10% of the migrating salmonids are infected and will probably die from this organism after entering salt water.

Since sampling began in 1981 the bacterial kidney disease organism, Renibacterium salmoninarum, has been detected by the fluorescent antibody test

in seven salmonid species caught in the open ocean off the coasts of Washington and Oregon. The bacterium has been found primarily in chinook salmon (11%) with lesions in 2.5% of these fish. This disease was also detected at levels ranging from 17% in coho salmon to 25% in chinook salmon seined from the Columbia River just before entering the estuary. Interpretation of these numbers suggests an even greater economic impact on Columbia River salmonid stocks than that proposed for C. shasta. Fertilized eggs from bacterial kidney disease infected parents examined after one month of incubation revealed the presence of bacteria with identical morphology to R. salmoninarum on or in the egg wall further reinforcing the proposed vertical transmission of this disease organism.

Infectious hematopoietic necrosis virus was recovered at the 67% level from seeded water samples supplemented with 1% fetal calf serum. Virus injected into unfertilized eggs survived for over two weeks; in eyed eggs the virus also replicated. Epizootics caused by IHNV occurred in two of the 8 separate groups of steelhead trout fingerlings held in LJV treated water at Round Butte Hatchery. Comparing these results to those in the vertical transmission experiment where none of the groups developed IHNV suggests that vertical transmission of IHNV, if it occurs, is a very infrequent or random event. On three occasions IHNV was detected in ovarian fluid samples after storage for 6-9 days at 4°C. No virus had been detected in these samples at spawning. This suggests the presence of an interfering substance, perhaps anti-IHNV antibody in ovarian fluid. This observation raises the possibility that IHNV is much more widespread throughout Columbia River Basin salmonid stocks than previously believed.

ACKNOWLEDGEMENTS

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Cooperators in this study are: the Portland General Electric Company, through their representative Mr. Don Ratliff, which owns Round Butte Hatchery and purchased the ultraviolet sterilization equipment; the personnel of the Oregon Department of Fish and Wildlife (ODFW) who operate Round Butte Hatchery, the ODFW also supplied the different stocks of fish and coordinated livebox placement; Dr. Warren Groberg, virologist of the ODFW who assisted in experimental design and sampling at Round Butte Hatchery ; Mr. Craig Banner of the ODFW who helped collect samples from the ocean; the United States Fish and Wildlife Service, Seattle Fisheries Research Center whose staff, directed by Dr. Dan Mulcahy, jointly sampled the steelhead trout at Round Butte Hatchery; the National Marine Fisheries Service personnel who helped in the collection of smolts from their Jones Beach seining facility; Dr. William Pearcy of the Oregon State University School of Oceanography and his research team who were responsible for the collection of juvenile salmonids from the ocean; and personnel fo the Army Corps of Engineers and the Grant County Public Utility District who helped coordinate the placing of liveboxes at selected Columbia and Snake River Dams.

INTRODUCTION

Infectious diseases are responsible for severe losses in Columbia River Basin salmonids. Successful propagation and enhancement of this fisheries resource requires control of these diseases. This study focuses on three serious fish pathogens: *Ceratomyxa shasta, Renibacterium salmoninarum,* and infectious hematopoietic necrosis virus. Knowledge gained will be useful in developing control measures for these pathogens and the application of these ideas will be of direct benefit in the successful rehabilitation of salmon stocks in the Columbia River Basin.

Ceratomyxa shata

Ceratomyxa shasta, a myxosporidan parasite of salmonid fish, is present in the Columbia River system and is devastating to certain stocks of fish (Sanders et al., 1970). Currently, the infectious stage of this histozoic parasite is known to exist in the mainstream of the Columbia River to the confluence with the Deschutes River. Tributary streams of the Columbia River also with the infectious stage are the Deschutes, Cowlitz and Willamette Rivers and Lacamas Creek and Lake (Johnson et al., 1979). Many areas in the Columbia Basin have not been examined for the infectious stage of *C. shasta* and changing environmental conditions within the basin may have extended the geographic range of the parasite. It would be valuable to know the exact range of *C. shasta,* especially to determine if infection can occur in tributaries where hatcheries exist or are being considered.

The impact of ceratomyxosis on anadromous fish entering salt water from the Columbia River is not understood. Fish infected with *C. shasta* in fresh

water may have a reduced level of survival in the ocean. To understand the total impact of this disease, it is necessary to define its effect on fish not only as they migrate through fresh water but also as they enter the marine environment.

Presently, there is no effective therapy for ceratomyxosis, but there is considerable evidence the disease can be controlled using C. shasta resistant stocks of fish (Zinn et al., 1977; Buchanan et al., 1983). To use this procedure, it is necessary to determine the resistance of fish stocks being reared.

The nature of the infectious stage of C. shasta, like most other myxosporidan parasites, is not known. Research concerning this parasite has been impeded because intermediate hosts and/or the infectious stage have not been described. Recently, there have been advances in the determination of these parts of the life history of Myxosoma cerebralis, another myxosporidan infecting salmonid fish (Wolf and Markiw, 1984). Knowing the nature of the infectious stage of C. shasta would greatly facilitate research on the host-parasite relationship of this pathogen and give valuable information concerning effective control of the disease.

Materials and Methods

Experimental Animals

Ceratomyxa shasta susceptible rainbow trout (Salmo gairdneri) were obtained from Oak Springs and Roaring River Hatcheries and held at the Oregon State University Fish Disease Laboratory (OSU-FDL) or the Round Butte Hatchery Isolation Facility (RBH-IF). Both these facilities are supplied with water

which is free of fish pathogens. Salmonid stocks to be tested for resistance to ceratomyxosis were obtained from Oregon Department of Fish and Wildlife hatcheries. All stocks were transferred to and held at the OSU-FDL prior to, and after, exposure to the infective stage of C. shasta.

In 1983 salmonid smolts exposed to C. shasta during their migration down the Columbia River were obtained from collection facilities operated by the National Marine Fisheries Service at Jones Beach (RKm 75) on the Columbia River. These fish were transported to the RBH-IF and held in 3-ft circular tanks (10°C) for 180 days. Fish that died within 10 days after arrival at RBH-IF were considered handling mortality and not included in the results.

Exposure to Ceratomyxa shasta

Prior to possible exposure to the infectious stage of C. shasta all groups of fish were fed for two weeks a Oregon Moist Pellet diet containing 3% terramycin in the form of TM5 (Pfizer) as a prophylactic measure against bacterial fish pathogens (Udey et al., 1975). Feeding with this medicated diet resumed when the fish were returned to the laboratory after exposure.

All groups of fish were exposed in 0.074-m cylindrical aluminum liveboxes placed at selected locations. Exposure periods were seven days for rainbow trout used in the distribution studies during 1983. In 1984 these studies were repeated using a 14 day exposure. After exposure, all fish were transported to either the OSU-FDL or RBH-IF and held until termination 100-150 days later. The water temperatures at the OSU-FDL and RBH-IF are a constant 12 and 10°C, respectively.

All groups used in the stock resistance portion of this study were exposed five days to the infectious stage of C. shasta in the Willamette River

near Corvallis, Oregon. After exposure these fish were returned to the OSU-FDL and held until termination 100 days later. Fish from several salmonid stocks were also transported to the Marine Science Center Fish Disease Laboratory (MSC-FDL) after *C. shasta* exposure and held in salt water.

Detection of *Ceratomyxa shasta*

Dead fish were collected daily, and either necropsied fresh or frozen for later examination. At termination of each experiment all surviving fish were killed and examined for *C. shasta*. Wet mounts of intestinal scrapings were examined microscopically (400X magnification) and preparations containing spores of *C. shasta* were considered infected.

Spore Purification and Antisera Production

Spores of *C. shasta* were obtained by removing intestinal materials from infected fish. These preparations were placed on a 10-55% sucrose gradient layered over 70% sucrose and centrifuged using a swinging bucket rotor at 1731 x g for 45 min. Spore-containing layers collected were further purified by additional gradient preparations. Spores were washed repeatedly in phosphate buffered saline (pH 7.2, PBS) and stored in PBS.

Rabbit anti-*C. shasta* serum was prepared by injecting purified spore preparations which had been broken by 15,000 psi in a French pressure cell and emulsified in Freund's complete adjuvant. Each rabbit received 2 x 107 spores divided equally into the hind foot pads and subcutaneously in the intrascapular region. Antibody containing serum was harvested 4-6 weeks after administration and the immunoglobulins partially purified and fluorescein labelled as described by Banner et al. (1982).

Concentration of *Ceratomyxa shasta* Infective Stage

Water (up to 200 liters per experiment) from sources containing the infectious stage of *C. shasta* as shown by infections produced in susceptible fish, was differentially filtered using a molecular filtration unit (Pellicon cassette system, Millipore Corp., Bedford, MA) with a pore size of 0.45 µm. Materials concentrated by this procedure were intraperitoneally injected and introduced into the stomach of susceptible rainbow trout and examined with the anti-*C. shasta* fluorescent antibody conjugate (FAT).

Exposure of Oligochaetes to *Ceratomyxa shasta* Spores

The oligochaete *Tubifex* was examined as a possible intermediate host for *C. Shasta.* They were collected from a water source that was negative for the infective stage of *C. Shasta* and maintained in 68 liter tanks containing a sterile sand substrate with 12°C runing water. Infected viscera and spores of *C. shasta* were added to the tanks. After three months, susceptible rainbow trout were introduced into the tanks and observed for mortalities resulting from transmission of ceratomyxosis.

Results and Discussion

Geographic Range of *Ceratomyxa shasta* in the Columbia River Basin

In 1983 fish were exposed at selected sites on the Columbia River for a period of seven days in June and September (Table 1). Infections caused by *C.*

Table 1. Incidence of Ceratomyxa shasta in susceptible rainbow trout (Salmo gairdneri) exposed at selected dams in the Columbia River Basin during June and September 1983[1] and during June 1984[2].

Location and time of exposure	Number of fish recovered[3]	Number of fish infected with Ceratomyxa shasta[4]	Percent of fish infected with Ceratomyxa shasta
Columbia River			
Bonneville Dam			
June 1984	25	4	16
Dalles Dam			
June 1983	56	0	0
Sept. 1983	50	0	0
June 1984	48	25	52
John Day Dam			
June 1983	59	0	0
Sept. 1983	50	0	0
June 1984	40	0	0
McNary Dam			
June 1983	58	3	5
Sept. 1983	50	0	0
June 1984	87	5	6
Priest Rapids Dam			
June 1984	55	0	0
Snake River			
Ice Harbor Dam			
June 1983	50	0	0
Little Goose Dan			
June 1984	93	1	1
Deschutes River			
Pelton Dam			
Sept. I9835	96	28	29
June 1984	51	23	45

1. In 1983, groups were exposed for 7 days.

2. In 1984, groups were exposed for 14 days.

3. Number of fish exposed minus handling mortalities.

4. All experimental groups were terminated and examined 120 days after initial exposure.

5. This group was exposed for 23 days.

shasta were found at a 5% level in fish exposed during June at McNary Dam. Infections were also found at a 29% level in fish exposed at Pelton Dam on the Deschutes River as a positive control. The disease was not found in fish exposed at the Dalles and John Day Dams on the Columbia River, or at Ice Harbor Dam on the Snake River during either exposure period.

During 1984 the exposure period was increased to 14 days. Again, infections were found at Pelton and McNary Dams during June at levels of 45% and 5.7% respectively. Infections were also found at a 16% level at Bonneville Dam, and at a 52% level at the Dalles Dam on the Columbia River, and at a 1.1% level at Little Goose Dam on the Snake River. The disease was not found at John Day or Priest Rapids Dams on the Columbia River (Table 1).

Susceptible rainbow trout were exposed in four tributaries of the Columbia River in 1983 (Table 2). Infections caused by C. shasta were not found in fish recovered from any of these sites.

The presence of infection at McNary Dam in 1983 and 1984 extends the range of the infectious stage of C. shasta in the Columbia River about 100 miles up-river. Finding the disease at Little Goose Dam in 1984 extends the range approximately 100 miles beyond that point, into the Snake River drainage. These observations greatly extend the range of C. shasta from that proposed by Johnson et al. (1979) in which the infectious stage was believed to enter the Columbia River from the Deschutes River.

Prevalence of Ceratomyxa shasta in Columbia River Salmonids

To test the resistance of Columbia River stocks of salmonids to infection by C. shasta, six hatchery stocks of salmonids were exposed in an area of the Willamette River known to harbor the infectious stage (Table 3). Oak Springs

Table 2. Incidence of Ceratomyxa shasta susceptible rainbow trout (Salmo gairdneri) exposed for seven days in Columbia River tributaries during July and August 1983.

Location	Number of fish recovered[1]	Number of fish infected with Ceratomyxa shasta[2]	Percent of Fish Infected with Ceratomyxa shasta
Lookingglass Creek	33	0	0
Grande Rhonde River	18	0	0
Wallowa River	58	0	0
Imnaha River	28	0	0

1. Number of fish exposed minus holding mortalities.

2. All experimental groups were terminated and examined 120 days after initial exposure.

Table 3. Susceptibility of salmonid stocks exposed for five days to the infectious stage of *Ceratomyxa shasta* in the Willamette River.

Salmonid Stock	Number of fish recovered[1]	Number of fish Infected with *Ceratomyxa shasta*[2]	Percent of fish infected with *Ceratomyxa shasta*
August 1983 exposures			
Rainbow trout			
Oak Springs	38	28	74
Chinook salmon			
Carson	20	0	0
Imnaha	43	1	2
Lookingglass	49	1	2
Coho salmon			
Sandy River[4]	25	0	0
Steelhead trout			
Wallowa	47	5	11
September 1983 exposures			
Rainbow trout			
Oak Springs	43	31	73
Chinook salmon			
Upriver Brights	56	1	2
Steelhead trout			
Imnaha	49		2

1. Number of fish exposed minus handling mortalities.

2. All experimental qroups were terminated and examined at 120 days after initial exposure.

3. Positive control.

4. Negative control

rainbow trout were used as a positive control because of their susceptibility to infection, and Sandy River coho salmon were used as a negative control because of their resistance. Four stocks of upriver chinook salmon, the Carson, Imnaha, Lookingglass, and upriver brights and one stock of upriver steelhead trout from the Imnaha all proved highly resistant, with levels of infection not exceeding 2%. The other upriver steelhead trout stock from the Wallowa River was more susceptible to ceratomyxosis with a 11% level of infection.

Salmonid smolts were collected periodically by beach and purse seines from the lower Columbia River between May 20 and September 8, 1983. Ceratomyxa shasta was present at levels ranging from 1 to 24% in those chinook salmon caught by beach seine (Table 4). Five percent or less of those collected during May and June contained spores of C. shasta; in contrast, five of the six groups from July through September had infectious rates of 12 to 24%. Infections at levels ranging from 5 to 33% were found in fish from 10 of the 11 groups of chinook salmon caught by purse seine (Table 5). Smaller numbers of steelhead trout and coho salmon were taken; 12 and 5%, respectively, of these species were infected (Tables 6 and 7).

While many of the upriver salmonids are considered resistant or only slightly susceptible to infection, these conclusions were drawn from experiments where the exposure period ranged from 3-7 days. Longer exposure periods may result in a higher prevalence of infection as suggested by results from smolts seined out of the lower Columbia River. Extension of the known range of the infective stage of C. shasta to Little Goose Dam on the Snake River also means upriver salmonids are exposed to this disease agent for a much longer period than prevlously recognized. Dawley et al. (1984) calculated that during 1983 the average migration rate for yearling chinook

Table 4. Prevalence of Ceratomyxa shasta in chinook salmon (Oncorhynchus tshawytscha) smolts beach seined from the Columbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected (1983)	Number Collected	Holding Mortalities	Mortalities infected with C. shasta	Percent of fish collected infected with C. shasta[2]
May 20	91	3	3	3
27	81	6	1	1
June 3	75	8	2	3
10	53	1	1	2
17	65	36	3	5
24	130	21	3	2
July 1	113	24	17	15
15	141	46	17	12
29	68	33	2	3
Aug. 12	109	36	13	12
26	34	20	8	24
Sept. 8	112	39	25	22
Totals	1072	273	95	8.9

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined and none were infected with Ceratomyxa shasta

Table 5. Prevalence of Ceratomyxa shasta in chinook salmon (Oncorhynchus tshawytscha) smolts purse seined from the Columbia River (RKm 75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected (1983)	Number collected	Holding mortalities	Mortalities infected with C. shasta	Percent of fish collected infected with C. shasta[2]
May 20	9	5	3	33
27	37	6	2	5
June 10	58	17	0	0
17	47	18	4	9
24	98	25	8	8
July 1	128	46	21	16
15	43	38	5	12
29	47	33	11	23
Aug. 12	47	45	4	9
26	21	18	1	5
Sept. 8	14	12	2	14
Totals	549	263	61	11.1

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined and none were infected with Ceratomyxa shasta.

Table 6. Prevalence of Ceratomyxa shasta in coho salmon (Oncorhynchus kisutch) smolts purse seined from the Colunbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected (1963)	Number Collected	Holding Mortalities	Mortalities infected with C. shasta	Percent of fish collected infected with C. shasta[2]
May 20	82	13	4	5
27	38	4	3	8
June 3	23	1	0	0
10	5	4	1	20
17	15	2	0	0
24	12	2	1	8
July 1	1	0	0	0
15	1	0	0	0
Aug. 26	2	0	0	0
Totals	179	26	9	5.0

1. Held in 3-ft circular tanks at a constant water temperature of 10°C

2. All fish alive at termination were examined and none were infected with Ceratomyxa shasta.

Table 7. Prevalence of Ceratomyxa shasta in steelhead trout (Salmo gairdneri) smolts purse seined from the Colunbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected (1983)	Number collected	Holding mortalities	Mortalities infected with C. shasta	Percent of fish collected infected with C. shasta[2]
May 20	11	5	2	18
27	13	4	0	0
June 3	45	13	6	13
10	4	2	0	0
24	1	1	1	100
July 1	1	1	0	0
Totals	75	26	9	12.0

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C

2. All fish alive at termination were examined and none were infected with Ceratomyxa shasta.

salmon and steelhead trout was 18 and 35 Km/day in the Columbia River. This means that the exposure period for these fish to C. shasta infective stages while migrating from Little Goose Dam to the Columbia River Bar would be 35 and 18 days, respectively.

Chinook salmon purse seined from the lower Columbia River were primarily yearlings, many of which would have came from these upriver locations. The 11% infection rate in these fish suggests the 2% level of infection from the four upriver chinook salmon stocks may underestimate the prevalence of ceratomyxosis in migrating salmonids. Similar conclusions can be made with the small numbers of steelhead trout collected. Coho salmon come chiefly from lower Columbia River locations giving them less exposure to the infective stage as the 5% level of infection suggests.

Effects of Salt Water on Fish Infected with Ceratomyxa shasta

Alsea steelhead trout and Big Creek coho salmon were exposed to the infectious stage of C. shasta in the Willamette River for three days. Half of each group were then transferred to salt water and half were held in fresh water. Of the Alsea steelhead trout 100% of those held in fresh water died of C. Shasta, and 88% of those transferred to salt water died of ceratomyxosis. None of the Big Creek coho salmon transferred to salt water died of C. shasta and only one fish held in fresh water developed spores, and these spores were found in a muscle lesion rather than in the intestinal tract.

Thus Alsea steelhead trout smolts exposed to the Infectious stage of C. shasta and held in salt water died at a similar rate as their counterparts held in fresh water, and Big Creek coho salmon held in either salt water or fresh water following exposure, were resistant to the disease. This indicates

that disease progress continues in salt water the same as in fresh water once the fish has become infected, and that fish which are resistant to the disease if they remain in fresh water will not develop an infection when they enter salt water. This is important in forming a model of infection for migrating salmonids.

Investigation into the Nature of the Infectious Stage of *Ceratomyxa shasta*

Antisera to *C. shasta* was labelled and evaluated for fluorescence and specificity. Smears of whole spores were used in evaluating fluorescence, which proved adequate for diagnosis. However when smears of river water seeded with spores were examined, the antisera was not sufficiently specific to differentiate between spores and non-spore material. Because one of the intended purposes of developing antisera to *C. shasta* was to identify prespore stages, specificity is important. To develop more specific antisera, alternate regimes for injection of spores into rabbits will be examined, along with the development of different spore preparations for injection.

Water was differentially filtered and examined for the presence of *C. shasta* using fluorescent antibody techniques and bright light microscopy. Two spores of the parasite were found in samples of filtered water, which is the first report of a spore being isolated from a natural water supply. Susceptible salmonids were also exposed to portions of this concentrate. *Ceratomyxa shasta* infections developed in fish exposed to two separate samples, showing that the infectious agent survives the filtration and concentration methods.

The technique of micromanipulation is also being used in conjunction with molecular filtration and concentration to isolate single cells which can be

injected into fish. In addition to injecting single organisms, whole samples of the concentrate are being injected and administered directly into the stomach of test fish. We hope that by combining molecular filtration and concentration with fluorescent antibody techniques and micromanipulation we may discover the nature of the infectious stage of C. Shasta.

The presence of an intermediate host in the life cycle of C. shasta has been suggested. Because Tubifex has been implicated in the life cycle of the myxosporidan Myxosoma cerebralis (Wolf and Markiw, 1983), we decided to examine its role in the infectivity of C. shasta Tubifex were incubated with infected viscera and spores of C. shasta for three months. Susceptible fish were then exposed to the Tubifex. No infections developed over a nine month exposure period, so it appears that Tubifex does not play the same role in the life cycle of C. shasta that it does in the life cycle of M. cerebralis. experiment is currently being repeated with some modifications. In addition to exposing fish to Tubifex which have been incubated with spores, the water above the oligochaetes will be examined for any free-living organisms that may have been shed, and the Tubifex themselves will be examined.

Renibacterium salmoninarum

Renibacterium salmoninarum, the causative agent of bacterial kidney disease (BKD) of salmonid fish, is widespread among hatchery reared fish (Fryer and Sanders, 1981). The disease is recognized as one of the major bacterial infections of salmon and problems caused by BKD extend throughout hatcheries which are operated in the Columbia River Basin. The disease is responsible for chronic mortality during the fresh water stage of the fishes' life cycle. Recent data indicate that mortality continues, perhaps at a higher rate, when fish enter salt water (Banner et al., 1983). Much of the mortality of salmon in the ocean could be a result of BKD, a disease contracted in fresh water. It is important to demonstrate the incidence of this disease among populations of salmonids and determine its effect on the animals as they enter sea water.

The disease is particularly severe because, at present, there are no effective methods for its control. The organism occurs intracellularly and therefore resists treatment by chemotherapeutants. The formulation of effective control methods is predicated upon understanding the organism, its pathogenesis, and especially the methods of its transmission. Because the organism is not easily combated after infection occurs, methods of control depend on knowledge concerning transmission of R. salmoninarum.

Although it has been generally accepted that there is only one antigenic type of R. salmoninarum (Bullock et al., 1974), there has been limited experimentation done to compare strains of this organism antigenically. Unpublished observations indicate there may be more than one antigenic type and that cross reactions may occur with bacteria from other genera. These observations have implications on vaccine development and on serological

methods used for disease diagnosis. Comparisons of antigenic composition are necessary to ensure that efficacious vaccines might be developed and that accurate disease diagnosis be made.

Materials and Methods

Experimental animals

Salmonids of different year classes were purse seined from the ocean off the coast of Washington and Oregon as part of a project conducted by the School of Oceanography at Oregon State University. About 3000 individual fish have been collected during the May through September sampling periods from 1981 through 1983. The same salmonids collected from the Jones Beach sampling area and transported to RBH-IF as part of the *C. shasta* survey were also examined for *R. salmoninarum* infections. As with the *C. shasta* results, all fish that died within 10 days after arrival were considered handling deaths and not included in the data.

Eggs from *R. salmoninarum*-infected, spawning chinook salmon were fertilized, incubated and hatched at the OSU-FDL. These eggs and resulting fry were examined for *R. salmoninarum* by FAT at selected intervals during development.

Antisera Production and Fluorescent Labelling

Rabbit anti-*R. salmoninarum* was prepared by injecting 10 bacterial cells emulsified in Freund's complete adjuvant (1:l). Equal volumes (0.2 ml) were injected into each hind foot pad and subcutaneously in the intrascapular

region. Antibody containing serum was harvested 30 days later and the immunoglobulins partially purified and fluorescein labelled as described by Banner et al. (1982).

Production of Monoclonal Antibodies

A modification of the method described by Oi and Herzenberg (1980) was used to produce hybridomas secreting antibody against three strains of R. salmoninarum. Strains used were Lea-l-74 ATCC 3320gT, RB-1-73 and K-50. Briefly, lymphocytes harvested from mice immunized with R. salmoninarum were fused with SP2 mouse myeloma cells in medium containing polyethylene glycol. After incubation in selective medium, each well of the tissue culture plate containing visible hybridoma colonies was tested for anti-R. salmoninarum antibody. Hybridoma cultures producing the desired antibody were expanded to be frozen in storage or cloned.

Enzyme-linked Immunosorbent Assay (ELISA)

Initially the indirect ELISA described by Voller et al. (1979) was used to screen for hybridomas producing anti-R. salmoninarum antibody. To increase sensitivity and obtain consistent results different ELISA assay procedures were tested. For this reason the double-sandwich indirect ELISA described by Mondallal et al. (1984) was adopted. Renibacterium salmoninarum cells were attached to 96-well polystyrene microtiter plates (Immulon) which were previously washed with rabbit anti-R. salmoninarum antibody. Test serum or supernatant from cultures containing a hybridoma was added to the wells then incubated with peroxidase-conjugated anti-mouse antibody. A positive culture

was detected by a visible color reaction following addition of 0-phenylene diamine substrate.

Results and Discussion

Prevalence of BKD in Ocean and Columbia River Salmonids

Kidney smears taken from salmonids captured in the open ocean off the coasts of Oregon and Washington were examined by PAT for presence of R. salmoninarum (Table 8). Since sampling began in 1981 positive kidney smears have been obtained from seven salmonid species. More than 11% of the chinook salmon caught have been infected and, in addition, BKD lesions have been seen in 2.5% of these fish and several have appeared unhealthy at capture. Lesions of BKD were also seen in a small number (0.3%) of the coho salmon captured.

Significant levels of R. salmoninarum were also detected in smolts seined from the Columbia River just prior to their entering the estuary. With the beach seined chinook salmon 41% of the mortalities that occurred during the 180 day holding period were infected with BKD; however, had the holding period been only 100 days 80% of the mortalities would have been infected with BKD (Table 9). Similarily with the purse seined chinook salmon, 71% of the mortalities were positive for BKD at 100 days (Table 10). Results with the coho salmon and steelhead trout followed a similar pattern (Tables 11 and 12).

The disease incidence varied widely between groups; for example, no BKD was detected in any of the 53 beach seined chinook salmon on June 10. This contrasts to a 57% level in the 65 fish collected on June 17 (Table 9). Correlation of those data with hatchery releases and ocean survival could yield valuable information about the effect of BKD on a given salmonid

Table 8. Prevalence of Renibacteriun salmoninarum in juvenile salmonids captured in the ocean off the coasts of Washington and Oregon from 1981 through 1983.

Salmonid Species	Number examined	Number infected with R. salmoninarum	Percent infected with R. salmoninarum	Number fish with kidney disease lesions	Percent fish with bacterial kidney disease lesions
Chinook salmon	721	80	11.1	18	2.5
Chum salmon	197	6	3.0	0	0
Coho salmon	1882	56	3.0	6	0.3
Pink salmon	15	2	13.3	0	0
Sockeye salmon	24	1	4.2	0	0
Cutthroat trout	95	1	1.0	0	0
Steelhead trout	91	3	3.3	0	0

1. All fish were examined for Renibacteriun salmoninarum by FAT.

Table 9. Prevalence of *Renibacterium salmoninarum* in chinook salmon (*Oncorhynchus tshawytscha*) smolts beach seined from the Columbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected	Number collected	Holding mortalities	Mortalities infected with *R. salmoninarum*	Percent mortalities Infected with *R. salmoninarum*	Survivors after 180 days holding at Round Rutte Hatchery	Number survivors Infected with *R. Salmoninarum*[2]	Percent survivors infected with *R. salmoninarum*	Prevalence of *R. salmoninarum* in all fish collected
May 20	91	3	1	33	88	7	8	8
27	81	6	1	17	75	13	17	14
June 3	75	8	1	13	67	12	18	12
10	53	1	0	0	52	0	0	0
17	65	36	29	81	29	28	96	57
24	130	21	5	24	109	4	4	7
July 1	113	24	8	33	89	13	15	18
15	141	46	16	35	95	1	1	12
29	68	33	20	61	35	0	0	29
Aug. 12	109	36	8	22	73	1	1	8
26	34	20	6	30	14	7	50	21
Sept. 8	112	39	17	44	73	3	4	17
Totals	1072	273	112	41.0	799	89	11.1	18.8

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined by FAT for *Renibacterium salmoninarum*.

Table 10. Prevalence of Renibacterium salmoninarum in chinook salmon (Oncorhynchus tshawytscha) smolts purse seined from the Columbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility[1].

Date collected (1983)	Number collected	Holding mortalities	Mortalities infected with R. salmoninarum	Percent mortalities infected with R. selmoninarum	Survivors after 180 days holding at Round Butte Hatchery	Number survivors infected with R. salmoninarun[2]	Percent survivors infected with R. salmoninarum	Prevalence of R. salmoninarum in all fish collected
May 20	9	5	3	60	4	0	0	33
27	37	6	5	83	31	1	3	16
June 10	58	17	7	41	41	5	12	21
17	47	18	8	44	29	5	17	28
24	98	25	9	36	73	27	37	37
July 1	128	46	17	37	82	3	4	16
15	43	38	27	71	5	1	20	65
29	47	33	6	18	14	0	0	13
Aug. 12	47	45	5	11	2	0	0	11
26	21	18	6	33	3	0	0	29
Sept. 8	14	12	4	33	2	0	0	28
Totals	549	263	97	36.9	286	42	14.7	25.3

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined by FAT for Renibacterium salmoninarum.

Table 11. Prevalence of Renibacteruim salmoninarum in coho salmon (Oncorhynchus kisutch) smolts purse seined from the Columbia River (RKm75) and held 180 days at Round Butte Hatchery Isolation Facility .

Date collected (1983)	Number collected	Holdinq mortalities	Mortalities infected with R. salmoninarum	Percent mortalities infected with R. salmoninarum	Survivors after 180 days holding at Round Rutte Hatchery	Number survivors infected with R. salmoninarum[2]	Percent survivors infected with R. salmoninarum	Prevalence of R. salmoninarum in all fish collected
May 20	82	13	8	10	69	4	6	15
27	38	4	3	8	34	4	12	18
June 3	23	1	1	4	22	0	0	4
10	5	4	3	60	1	0	0	60
17	15	2	2	13	13	4	31	40
24	12	2	1	8	10	0	0	8
July 1	1	0	0	0	1	0	0	0
15	1	0	0	0	1	0	0	0
Aug. 26	2	0	0	0	2	0	0	0
Totals	179	26	18	69.2	153	12	7.8	16.8

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined by FAT for Renihacterium salmoninarum.

Table 12. Prevalence of Renibacterium salmoninarum in steelhead trout (Salmo gairdneri) smolts purse seined from the Columbia River (RKm75) and held 180 days at Round Rutte Hatchery Isolation Facility[1].

Date collected (1983)	Number collected	Holding mortalities	Mortalities infected with R. salmoninarum	Percent mortalities infected with R. salmoninarum	Survivors after 180 days holding at Round Butte Hatchery	Number survivors infected with R. salmoninarum[2]	Percent survivors infected with R. salmoninarum	Prevalence of R. salmoninarum in all fish collected
May 20	11	5	3	60	6	0	0	27
27	13	4	4	100	9	0	0	31
June 3	45	13	7	54	32	0	0	16
10	4	2	2	100	2	0	0	50
24	1	1	0	0	0			0
July 1	1	1	1	100	0			100
Totals	75	26	17	65.4	49	0	0	22.7

1. Held in 3-ft. circular tanks at a constant water temperature of 10°C.

2. All fish alive at termination were examined by FAT for Renibacteriun salmoninarum.

stock. Both ocean and Columbia River iuvenile samples indicate R. salmoninarum is widespread in many salmonid populations. Also the disease pathology often seen in ocean caught juveniles and the BKD mortality associated with holding Columbia River smolts reinforces the important economic impact of this disease on Columbia River Basin salmonid stocks.

Transmission of Renibacterium salmoninarum

Egg washings and kidney smears were collected from individual spawning adult spring chinook salmon at Round Butte Hatchery (RBH) during 1983 and 1984 (Table 13). The samples collected in 1984 were from fish which had received a single intraperitoneal injection of erythromycin (5 mg/lb) 30 days prior to spawning. This probably accounts for the reduced detection of R. salmoninarum; however, combining results from the kidney smears and egg washings would give a higher 36% figure (21 of 59). It should be noted that smears were also made from spleens of 20 of these fish. In these samples the bacterium was more common (12 of 20 or 60%), suggesting the spleen should be considered when examining for BKD in antibiotic treated fish.

Fertilized eggs from parents with BKD were incubated at the OSLJ-FDL. Examination by Gram stain of cryostat sections (16 µm thick) from samples of these eggs revealed the presence of bacteria with identical morphology as R. salmoninarum on or in the egg wall. Additional sections examined by FAT further supported the presence of R. salmoninarum at these locations. Evelyn et al. (1984) recently suggested that the pathogen is in the yolk of unfertilized water-hardened eggs, our observations extend these results by indicating the bacterium can still be found in fertilized eggs examined just prior to hatching after one month of incubation.

Table 13. Renibacterium salmoninarum in egg washings and kidney smears from spawning adult spring chinook salmon (Oncorhynchus tshawytscha) at Round Butte Hatchery.

	Egg Washings			Kidney Smears		
	Number Examined	Number positive for R. salmoninarum[1]	Percent positive for R. salmoninarum	Number examined	Number positive for R. salmoninarum[1]	Percent positive for R. salmoninarum
1983						
Second egg take	20	16	80	20	10	50
Third egg take	30	22	73	30	16	53
1984						
First egg take	60	13	22	59	13	22

1. All samples were examined by FAT for Renibacterium salmoninarum.

Monoclonal Antibodies

Eight fusions were done using lymphocytes from mice immunized with these different strains of *R. salmoninarum*. Hybridomas from fusions 2, 3 and 4 were lost because of bacterial contamination. Using indirect ELISA, 26% of the hybridomas tested were positive for anti-*R. salmoninarum* antibody production (Table 14). Of these, 22 were expanded for storage and/or cloned.

A major advantage of hybridoma production is the generation of a continuous source of homogeneous antibodies to a single antigenic determinant. It has been shown by this study that the production of monoclonal antibodies to *R. salmoninarum* is possible. These antibodies will be used to detect differences in antigenic composition of *R. salmoninarum* strains. Hybridomas will later be injected into mice to produce ascites fluid containing monoclonal antibodies against *R. salmoninarum,* providing a source of homogeneous antibody for immunodiagnostic techniques.

ELISA

Positive hybridomas were identified using the indirect ELISA described by Voller et al. (1979). However, results were inconsistent and of limited sensitivity. Mondallal et al. (1984) compared different ELISA procedures and found a greater number of hybridomas could be detected using the double-sandwich method. This method was tested and compared to other assay procedures. The titer detected in serum from a mouse immunized with *R. salmoninarum* was 2-4 times the titer detected by microagglutination or indirect ELISA (Table 15). This method will be modified and tested to determine whether *R. salmoninarum* antibody can be detected in fish serum and

Table 14. Summary of antibody producing hybridomas to Renibacterium salmoninarum

Fusion number	Antigen	Number of hybridomas tested	Nunber positive hybridomas	Nunber hybridomas stored	Nunber clones obtained
1	Lea-1-74	9	1	1	4
5	K-50	112	34	5	_1
6	RB-1-73	143	24	5	_1
7	Lea-1 -74	158	56	5	_1
8	RB-1-73	81	6	6	_1

1. Serum lot tested inadequate to support clone development.

Table 15. Comparison of Renibacterium salmoninarum antibody titers using three assay methods.

Method of assay	Mouse serum titer detected[1]	
	Mouse A	Mouse B
Microagglutination	400	200
Indirect ELISA	400	400
Double Sandwich ELISA	1600	800

1. Titers were determined from duplicate samples at each dilution.

may provide an improved immunodiagnostic technique for detection of R. salmoninarum infections.

Infectious Hematopoietic Necrosis Virus

Another nontreatable disease which occurs among Columbia River Basin salmonids is infectious hematopoietic necrosis virus (IHNV). This viral disease was once confined to limited locations within the basin, but has recently become more widespread and has been responsible for devastating losses among chinook salmon and steelhead trout (Groberg and Fryer, 1983). The magnitude of the problem caused by IHNV has increased and effective control of this disease has become tantamount to the successful propagation of anadromous fish at several Columbia River Basin hatcheries.

Because there are no efficacious chemotherapeutants or anti-IHNV drugs, management techniques which use methods of avoidance of the virus have been attempted for its control. Methods of avoidance that have been employed are the use of virus-free water for the rearing of fish or the propagation of eggs known to be from virus-free adults. The effectiveness of each of these methods has been equivocal and would be better understood if the method(s) of virus transmission were defined. There are reports which indicate IHNV can be transmitted either vertically and/or horizontally (Pilcher and Fryer, 1980; Mulcahy et al., 1982). Infections in the laboratory using water borne challenges (horizontal transmission) are easily accomplished in salmonid alevins. Vertical transmission has not been definitively demonstrated under laboratory conditions and although there are many emperical observations, the role of horizontal and vertical transmission of IHNV under natural conditions has not been determined. If the virus is transmitted vertically, the propagation of eggs from virus-free adults is a management technique which could be used to control IHNV. However if IHNV infects fish through the water, control of the disease is dependent on the use of virus-free water for

egg and fish propagation. If both vertical and horizontal transmission occurs, both types of control measures may be necessary.

Studies under controlled laboratory conditions in conjunction with large scale experimentation are necessary to define the mode of virus transmission and to accurately evaluate the method(s) of disease control. At RBH, IHNV was first detected in 1973. Since that time, attempts have been made to gain a better understanding of the etiological agent and the disease it causes. Although detailed studies have not been possible, some data concerning the incidence of IHNV in spawning adults and subsequent outbreaks of the disease are available (Table 16). Because of these data and because of the physical facilities of RBH, this site offers an opportunity for a concerted effort to determine the role both vertical and horizontal transmission play in IHNV infections and to determine methods for their control.

Materials and Methods

Virus Propagation and Detection

Chinook salmon embryo (CHSE-214) and epithelioma papillosum cyprini (EPC) cell lines were continuously cultured in Eagle's minimum essential medium (MEM) supplemented with fetal calf serum (10%), $NaHC0_3$ (0.075%), penicillin (100 i.u./ml) and streptomycin (100 µg/ml). The EPC MM growth medium was also buffered with Tris-hydrochloride (Sigma). Growth temperatures were 16°C for CHSE-214 cells and 22°C for EPC cells.

Plaque assay procedures were similar to Burke and Mulcahy (1980). Assays were performed using confluent EPC cell monolayers grown in either 2.0 or 4.5 cm^2 tissue culture multi-well plates (Linbro). Samples were filter

Table 16. Detection of Infectious Hematopoletic Necrosis Virus in adult summer steelhead trout (Salmo gairdneri) and epizootics in fry at Round Butte Hatchery, 1975-1983.

Year	IHNV in Brood (Percent positive)	Epizootics in fry	Groups lost/total	Total fry loss
1975	Negative	Yes	2/5	550,000
1976	Positive	Yes	1/4	182,000
1977	30	No		
1978	Positive	Yes	4/6	425,000
1979	21	No		
1980	44	Yes	5/16	144,000
1981	50	No		
1982	0	Yes	10/16	400,000[1]
1983	51[2]	Yes	2/24	90,000

1. An additional 70,000 fingerlings were kept even though IHNV was diagnosed.

2. Combined carrier rate of males and females tested.

sterilized (0.2 µm acrodisc, Gelman) diluted in either MEM (without fetal calf serum) or Hank's balanced salt solution (HBSS). Duplicate samples (0.05-0.1 ml) of each of the 10^{0}-10^{-2} dilutions were placed on monolayers in individual wells and allowed to adsorb for 60 minutes. Sample inoculum was removed from the wells after adsorption and l-l.5 ml of overlay medium containing 0.75-1% methyl-cellulose dissolved in double strength MEM (without fetal calf serum) was added. Following seven days of incubation at 16°C cells were fixed with formalin and stained with 1% crystal violet solution. Plaques were counted using a dissecting microscope at 10% magnification.

Ovarian and seminal fluids and tissue from the spleen were collected from spawning steelhead trout at RBH and held at 4°C while transported to the laboratory. Ovarian fluids were inoculated directly onto the cells. Seminal fluids were centrifuged at 2000 x g for 10 minutes at 4°C to separate the supernatant from the sperm cells and the supernatant was then inoculated directly onto the cells. Tissue samples were weighed and diluted (w/v) 1:l0 with HBSS and homogenized with a stomacher (Tekmar). The tissue homogenate was centrifuged at 2000 x g for 10 minutes at 4°C and supernatant fluid inoculated onto cells. All samples were stored at 4°C.

Initial gamete storage trials were conducted with 1983 brood spring chinook salmon at RBH. It was found that by placing eggs and sperm in separate plastic bags, inflating the bags with oxygen, and placing them at 4°C for up to six days prior to fertilization, survival to hatching (86.1%) was similar to production eggs (88.1%) fertilized at spawning (B. Nyara, personal communication).

Concentration of IHNV from Water

Tangential flow filtration was evaluated as a method to concentrate IHNV from water systems. Procedures followed were from the Millipore Corporation, Virus Concentration Manual (1980). The Pellicon cassette system used had a membrane exclusion size of 100,000 molecular weight.

Ten liter volumes of OSU-FDL water were held for at least 12 hours at 4°C before seeding with IHNV. Virus seeded water was treated by several methods prior to filtration: 1) stirred slowly for two hours before filtration, 2) immediately filtered, or 3) heat-inactivated fetal calf serum (l%, Hyclone) was added one hour prior to virus addition and filtration. Water seeded with virus was kept in an ice bath throughout each filtration period. Samples of the retentate, filtrate and backflushes were collected and virus recovery determined.

Fish Egg Inoculations

Unfertilized eggs from spawning IHNV-negative rainbow trout were injected (0.01 ml) with a viral suspension containing 1 x 10^4 plaque forming units (PFU) per egg. After injection the eggs were kept on a screened tray in flowing 12°C water at the OSU-FDL. Daily, five eggs were individually sampled for 10 days then on alternate days for 10 additional days to determine the survival of IHNV. The eggs sampled were weighed and homogenized 1:l0 (w/v) in HBSS. Each homogenate was centrifuged and the supernatant inoculated onto EPC cells. After a six day incubation at 16°C, the cells were fixed and stained and the plaques counted. A control group of eggs were injected with MEM only and handled in the same manner as those in the experimental group.

Fertilized steelhead trout eggs were injected with virus suspensions containing known concentrations of IHNV on days 18, 20, 22 and 24 of embryonic development. These eggs were allowed to hatch and any mortality after hatching was examined for signs of IHNV disease. Dead fry were also weighed, processed and assayed to determine if the virus survived and replicated after injection.

Design of IHNV Transmission Studies at RBH

Ultraviolet (UV) sterilization equipment (model 120L, Ultraviolet Technology, San Marcos, CA) was installed at RBH on a portion of the egg incubation water and on water supplying 16, 6-ft circular tanks used for rearing fingerlings. Based on manufacturers specifications and the flow rate used, the estimated minimum dosage of UV irradiation was 40,000 µ watt seconds per square centimeter. This level was maintained while the eggs and yolk-sac fry were in the incubation trays. After the fry were transferred to the 6-ft circular tanks those from the high and no-titer IHNV crosses were placed in water treated with UV at this same level, while the eight control groups of fry used to test horizontal transmission of IHNV were placed in water treated at 80,000 µ watt seconds per square centimeter.

The purpose of this two-part study was to determine if IHNV which has caused epizootics in steelhead trout at RBH since 1975 (Table 16), is introduced via the water supply (horizontal transmission) or is transmitted from the adults (vertical transmission) or if both routes of transmission occur. This virus is found in kokanee salmon (Oncorhynchus nerka) in the reservoir above the hatchery and in adult spring chinook salmon and steelhead trout returning to the hatchery.

In the experiment to test horizontal transmission of IHNV (Appendix A), tissue and sex fluid samples were taken from 160 individual steelhead trout (80 males and 80 females) divided into 10, 16 fish pools. Eggs and sperm from fish in eight of these pools were then divided into two subgroups. One subgroup was fertilized, water-hardened, incubated and reared in UV treated water and the other subgroup received untreated hatchery water for each of these steps. This procedure was repeated so eight paired subgroups from the same adults were reared. After hatching, the fish were moved to 16, 6-ft circular tanks (eight groups continued on UV treated water and eight continued on non-treated water) until they reached 3-5 grams in size. Then all individual groups, totaling 237,000 fish, were combined and released.

The second portion of the IHNV study at RBH was designed to test vertical transmission of the disease (Appendix A). Sex products from all adult steelhead trout spawned were stored separately in plastic bags and placed at 4°C until virus tests were completed. Selected crosses were then made with gametes from high-titer IHNV positive parents and no-titer IHNV negative parents. These crosses resulted in eight groups each containing gametes from eight females and three males. At the time of crossing, sex fluids were collected to determine the virus titer of the pooled groups and to ensure that after the nine day storage period IHNV positive groups were still positive and negative groups were still negative. All water used throughout this experiment from fertilization to rearing was treated with UV light. After hatching, fry from IHNV positive parents were reared in 4, 6-ft circular tanks and those from IHNV negative parents in four additional tanks. At the 1.5 to 2.0 gram size, the 64,000 fish from the high-titer IHNV positive parents were destroyed and the 64,000 fish from the no-titer IHNV negative parents were released.

Eggs taken from high-titer IHNV positive parents and no-titer IHNV negative parents at RBH were also held at the OSU-FDL and fertilized and water-hardened in a virus suspension containing 10^5 PFU/ml. A portion of these eggs were disinfected with iodophor (100 ppm for 15 minutes) after water hardening. These groups were incubated, sampled, and hatched at the OSU-FDL to determine if virus survives on the egg and if progeny become infected with IHNV.

Results and Discussion

Recovery of IHNV by Molecular Filtration

Initial attempts to recover known amounts of IHNV from seeded OSU-FDL water using the Millipore Pellicon cassette system with a 100,000 nominal molecular weight limit were disappointing. Virus recoveries from seeded water samples after 44 to 230-fold concentrations (Runs l-7) ranged from 0 to 21% (Table 17). Results of plaque assays performed on samples collected prior to Eiltration (Runs l-3) suggested that large quantities of IHNV were lost while stirring; however, immediate filtration after virus addition (Runs 4-7) failed to improve recovery (Table 17). These results indicated need for addition of a stabilizing agent to the water before filtration.

Mathes et al. (1977) and Gangemi et al. (1977) have reported over 90% recovery by molecular filtration of feline leukemia virus and arenaviruses, respectively, from tissue culture fluid containing fetal calf serum. Also, Berman et al. (1980) by membrane pretreatment with flocculated beef extract greatly improved recovery of poliovirus from distilled water samples. Supplementation of the seeded water with 1% fetal calf serum (Run 8, 118-fold

Table 17. Recovery of virus from water seeded with a known concentration of Infectious Hematopoietic Necrosis Virus.

Filtration run and Handling	Concentration of virus seeded in water (PFU)[1]	Final virus concentration (PFU/ml)	Retentate volume (mls)	Infective particles in retentate (PFU)	Percent virus recovery	
					Retentate	Filtrate
Stirred 2 hours						
1	2.9×10^9	2.9×10^5	120	6.0×10^8	21	–[2]
2	2.0×10^9	2.0×10^5	185	0	<1	<1
3	1.93×10^9	1.93×10^5	225	1.04×10^8	5	<1
Filtered Immediately						
4	1.65×10^9	1.65×10^5	170	1.97×10^8	12	<1
5	1.2×10^9	1.2×10^5	140	1.34×10^8	11	<1
6	1.1×10^3	1.1×10^{-2}	140	0	<1	<1
7[1]	1.26×10^6	1.26×10^2	130	0	<1	<1
1% serum added						
8	1.96×10^6	1.96×10^2	85	1.25×10^6	67	<1

1. All volumes filtered were 10 liters except filtration run number seven which was 30 liters.

2. No sample was collected.

concentration) resulted in a greater than three-fold increase in virus recovery over the best previously observed (Table 17). We do not know whether fetal calf serum stabilized the virus or blocked virus adsorption sites in the membrane. Still some 30% of the virus was unaccounted for and not present in the filtrate. Further development of procedures to stabilize the virus and/or to prevent virus adsorption to the membrane should enable additional improvements in recovery.

Survival of IHNV in Fish Eggs

Injected IHNV survived for over two weeks in unfertilized rainbow trout eggs. During this period the virus titer decreased at a slow but steady rate from 5×10^4 PFU's/egg on day one to 1.7×10^3 PFU's/egg on day 16 (Figure 1). Viral plaques was still detected from eggs sampled on days 18 and 20; however, the numbers were no longer statistically significant.

In comparison to the gradual decline of IHNV in unfertilized eggs the data from the injection of eyed eggs show not only survival but replication (Table 18). Signs of IHNV disease were not seen in the fry, perhaps, because few survived for longer than one week after hatching; however, virus titers often several logs higher than in the original inoculum, indicated rapid viral replication and disease development.

Steelhead trout eggs from RBH were incubated at the OSU-FDL and sampled daily for IHNV. One egg sampled on day 16 from the group with high-titer IHNV parents was positive for the virus. The results from egg inoculations plus the detection of IHNV associated with an egg from known positive parents suggest vertical transmission; however, the finding of only one PFU ($\geq 4 \times 10^2$ virus particles) from a single egg indicates it is an uncommon event.

Figure 1. Virus recovered from unfertilized rainbow trout eggs, injected with infectious hematopoietic necrosis virus.

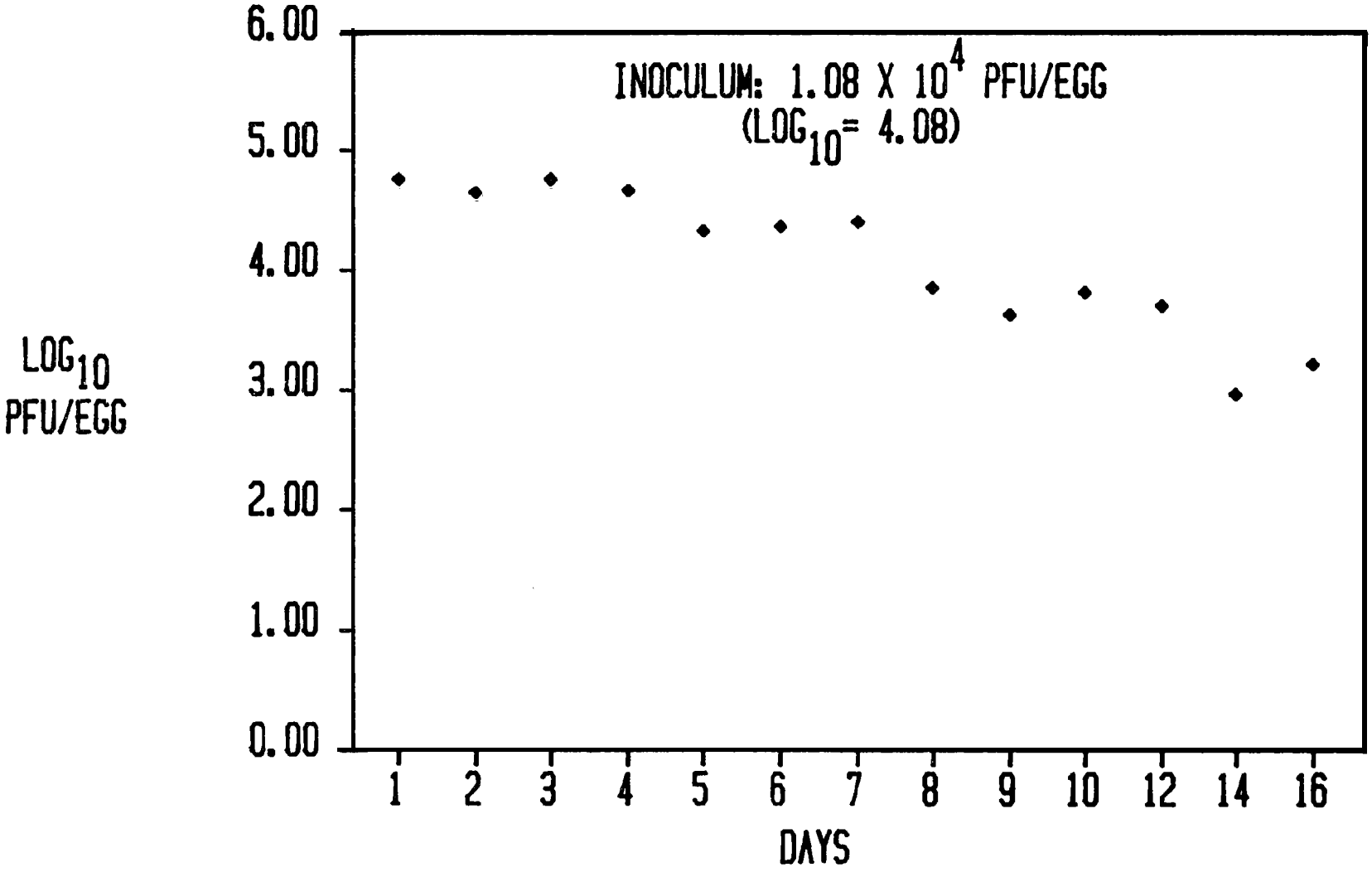

Table 18. Infectious Hematopoietic Necrosis Virus recovered from steelhead trout (Salmo gairdneri) fry that hatched from eggs injected with virus on days 18, 20 and 22 of embryonic development.

Inoculum virus titer:
Day 18 - 1.90 x 10^4 PFU/eqg
Day 20 - 2.33 x 10^4 PFU/egg
Day 22 - 4.20 x 10^4 PFU/egg

Mortalities Sampled	Day 18 Virus Titer	Day 20 Virus Titer	Day 22 Virus Titer
1	>4 x 10^5	7.2 x 10^4	<400
2	1.3 x 10^4	<400[1]	<400
3	>4 x 10^5	5.4 x 10^6	>4.0 x 10^6
4	9.2 x 10^7	>4.0 x 10^5	1.4 x 10^6
5	1.7 x 10^9	1.1 x 10^7	<400
6		<400	1.2 x 10^7
7		3.6 x 10^6	4.6 x 105
8		>4.0 x 10^5	>4 x 10^5
9		>4.0 x 10^5	<400
10		>4.0 x 10^5	>4.0 x 10^5
11		>4.0 x 10^5	>4.0 x 10^5
12		>4.0 x 10^5	>4.0 x 10^5
13		>4.0 x 10^5	
14		>4.0 x 10^5	
15		>4.0 x 105	

1. This value represents the minimum number of virus particles that must be present in the sample for the detection of one PFU.

Transmission of IHNV at RBH

Adult steelhead trout from the first spawning at RBH had a IHNV carrier rate of 54% in the females and 29% in the males. In the second spawning the carrier rate was 21% in the males and 60% in the females. Results of the vertical transmission experiment in which none of the eight groups (four high-titer groups and for no-titer groups) on UV treated water developed IHNV disease, indicate vertical transmission did not occur. However, comparing these results with the production lots, in which two of the eight groups receiving UV treated water developed the disease, further suggests that vertical transmission may represent a random and infrequent event.

A surprising and potentially very significant observation that could cause extensive modification of sampling procedures for IHNV, was made when crossing the selected positive and negative gametes stored for nine days at 4°C. Results from these pooled samples showed increased titers in three of the four positive groups, but most importantly IHNV was now detectable in groups E-H, groups in which the virus had not been detected at spawning (Table 19).

Comparing the mean IHNV titers of female gametes at spawning with those after pooling show a doubling in titer for samples From group D and log increases for groups A and B. The group C titer had decreased by more than a log. From one to three logs of virus was detectable in Groups E-H compared to their initial negative or no-titer status. Seminal fluid samples from all eight groups displayed no differences between samplings (Table 19).

The detection of IHNV after storage of ovarian fluid was repeated two more times with steelhead trout from RBH. In a second experiment, ovarian

Table 19. Infectious Hematopoietic Necrosis Virus titers of sex fluids from steelhead trout (Salmo gairdneri) parents and titers in these sex fluids when pooled after being stored separately for nine days at 4°C.

Group	Ovarian Fluid Mean Titer[1]	Ovarian Fluid Pooled Titer	Seminal Fluid Mean Titer[2]	Seminal Fluid Pooled Titer
A	2.01×10^6	2.11×10^7	1.37×10^3	5.1×10^3
B	1.35×10^6	4.92×10^7	1.47×10^3	1.9×10^2
C	7.35×10^6	6.5×10^5	8.0×10^1	1.4×10^2
D	2.82×10^6	4.16×10^6	7.0×10^1	5.0×10^1
E	0	1.0×10^1	0	0
F	0	2.3×10^3	0	0
G	0	1.2×10^2	0	0
H	0	1.3×10^2	0	0

1. Mean titer of eight parental fish.

2. Mean titer of three parental fish.

fluid samples were taken and assayed for IHNV beginning at day 0 and continuing through day 18 with a two day sampling interval. Results showed low-titer ovarian fluid samples increasing in titer and also initial no-titer samples developing low-titers. These results suggest the presence of an interfering agent,perhaps anti-IHNV antibody in the ovarian fluid initially neutralizes any IHNV present but with storage the reaction is reversed and virus released.

Fish Disease Database

Columbia River Basin fish pathologists have been contacted and from their recommendations a fish examination report (Appendix B) has been formulated and made available to fish health managers in each of the cooperating agencies. The information collected will be computerized and stored so it can be readily retrieved as required. This information will be used to publish periodic epidemiological reports similar to those used for human and veterinary medicine.

SUMMARY AND CONCLUSIONS

During fiscal year 1983, Bonneville Power Administration funded a study concerning the epidemiology and control of three infectious diseases of salmonids in the Columbia River Basin. These serious fish pathogens are: Ceratomyxa Shasta, the causative agent of ceratomyxosis, Renibacterium salmoninarum, the causative agent of bacterial kidney disease and the viral disease agent Infectious Hematopoietic Necrosis Virus.

Ceratomyxa shasta

The presence of ceratomyxosis in rainbow trout exposed at McNary Dam in 1983 and 1984, and at Little Goose Dam in 1984 extends the range of this disease about 200 miles upriver and into the Snake River drainage. Prior to our observations the infectious stage of C. shasta was believed to enter the Columbia River from the Deschutes River. These results mean upriver salmonids are exposed to this disease agent for a much longer period than previously believed.

There is no effective therapy for ceratomyxosis, but there is considerable evidence the disease can be controlled using C. shasta resistant stocks of salmonids. Four stocks of upriver chinook salmon the Carson, Imnaha, Lookingglass and upriver brights and one stock of upriver steelhead from the Imnaha all proved highly resistant (<2%) after a five day exposure to the infective stage of C. shasta. The other upriver steelhead stock from the Wallowa River was more susceptible (11%).

To determine the impact of ceratomyxosis on anadromous smolts entering salt water they were collected periodically by beach and purse seine from the

lower Columbia River (RKm75) between May 20 and September 8, 1983. Nine percent of the beach seined chinook salmon and 11, 5 and 12 percent, respectively, of the purse seined chinook and coho salmon and steelhead trout were infected. The chinook salmon and steelhead trout purse seined from the lower Columbia River were primarily yearlings, many of which would have come from upriver locations. The 11 and 12% infection rates in these fish suggests that with prolonged exposure to the infective stage of C. shasta even resistant stocks will become infected. Migration from Little Goose Dam to the Columbia River bar requires at least 20 days. Our experiments have also indicated ceratomyxosis progresses in salt water at the same rate as in fresh water once the fish has become infected. These observations mean that approximately 10% of the salmonids entering the estuary after migrating down the Columbia River are infected with C. shasta and that once infected these fish will probably continue to die after entering the ocean.

Attempts to transmit ceratomyxosis to susceptible fish by exposing them to Tubifex were unsuccessful suggesting this oligochaete does not play the same role in the life cycle of C. shasta as proposed for the life cycle of Myxosoma cerebralis.

Renibacterium salmoninarum

Since sampling began in 1981, kidney smears positive for R. salmoninarum have been obtained from seven salmonid species caught in the open ocean off the coasts of Oregon and Washington. The bacterium has been found primarily in chinook salmon (11%) with lesions in 2.5% of these fish. Significant levels of R. salmoninarum were also detected in smolts seined from the Columbia River just before entering the estuary. These observations further

demonstrate the economic impact of this disease on Columbia River salmonids stocks. The capture of clinically diseased fish in the open ocean is a rare event, and the presence of R. salmoninarum caused lesions in these fish is especially valuable information, indicating the existence of an ongoing open ocean epizootic.

Examinations by Gram stain and the fluorescent antibody test of cryostat sectioned fertilized eggs from bacterial kidney disease infected parents revealed the presence of fluorescing bacteria with identical morphology as R. salmoninarum on or in the egg wall. These observations by indicating the bacterium can still be found in fertilized eggs after one month of incubation further demonstrate this disease is vertically transmitted.

Infectious Hematopoietic Necrosis Virus

Recovery of IHNV from seeded water samples demonstrated that molecular filtration could be used to analyze hatchery water supplies for the presence of this virus.

Injected IHNV survived for over two weeks without replication in unfertilized eggs; whereas, in eyed eggs the virus not only survived but replicated and caused deaths among fry that hatched from these eggs.

Carrier rates of IHNV in steelhead trout spawned at Round Butte Hatchery ranged from 54-60% in females and 21-29% in males. In the transmission experiments at Round Butte Hatchery, IHNV occurred in two of the eight groups receiving LJV treated water and was not detected in any of the high-titer or no-titer crosses. However the virus was detected in one egg from high-titer parents after 16 days of incubation. These results suggest that vertical transmission of IHNV, if it occurs, is a very infrequent and rare event.

On three occasions IHNV was detected in ovarian fluid samples after storage for six to nine days at 4°C. No virus had been detected in these samples when collected at spawning. This suggests the presence of an interfering substance, perhaps anti-IHNV antibody, in the ovarian fluid. Routine sampling for IHNV requires the processing of tissues and sex fluids taken at spawning; however, if interfering substances are present as suggested by these experiments then the virus would not be detected. These findings raise the possibility that IHNV is much more widespread than previously thought and also that sampling procedures for this virus will require major modifications.

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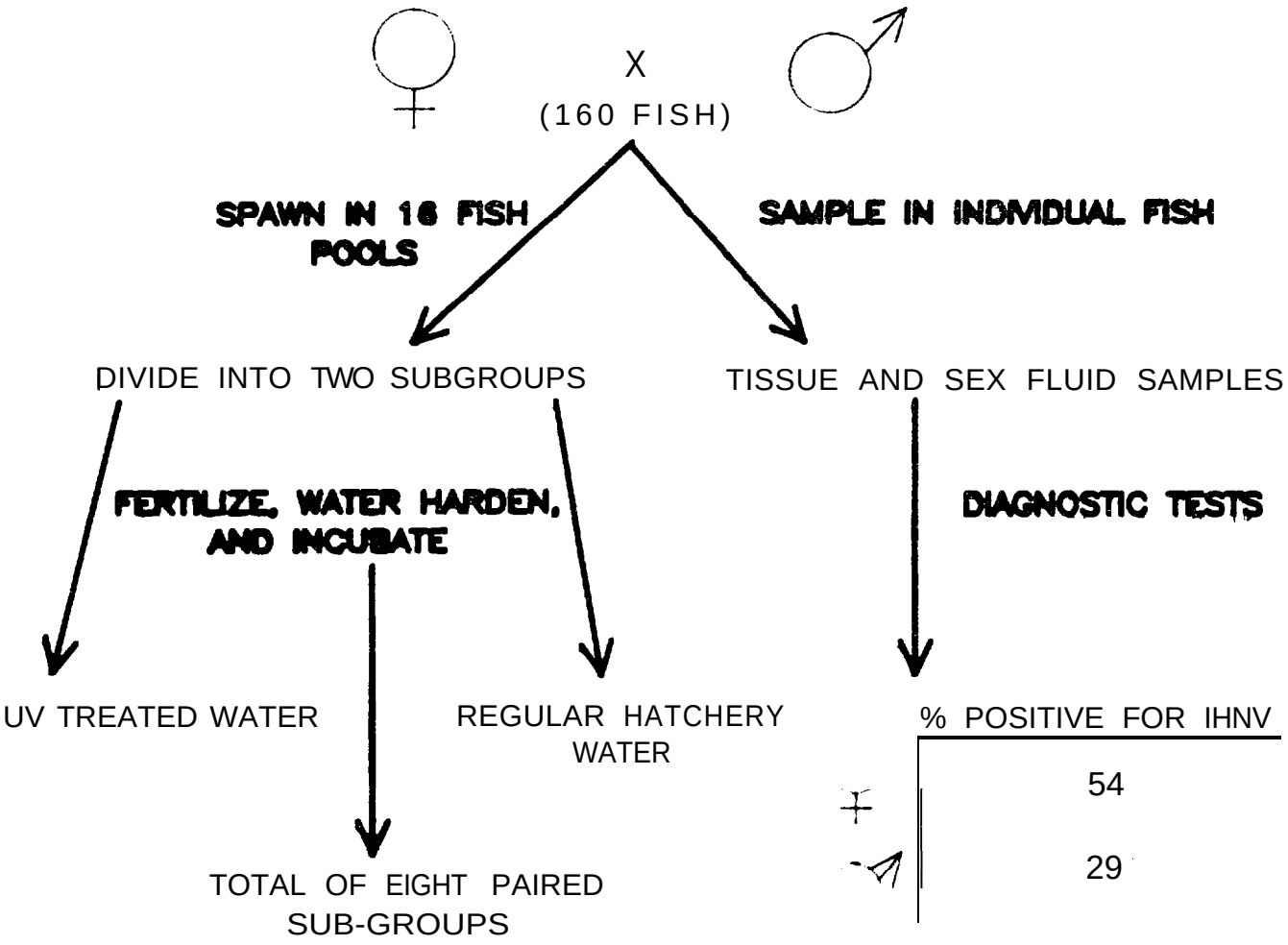
APPENDIX A

FLOW SHEETS OF ROUND BUTTE HATCHERY IHNV EXPERIMENTS

PRODUCTION CROSSES/HORIZONTAL TRANSMISSION

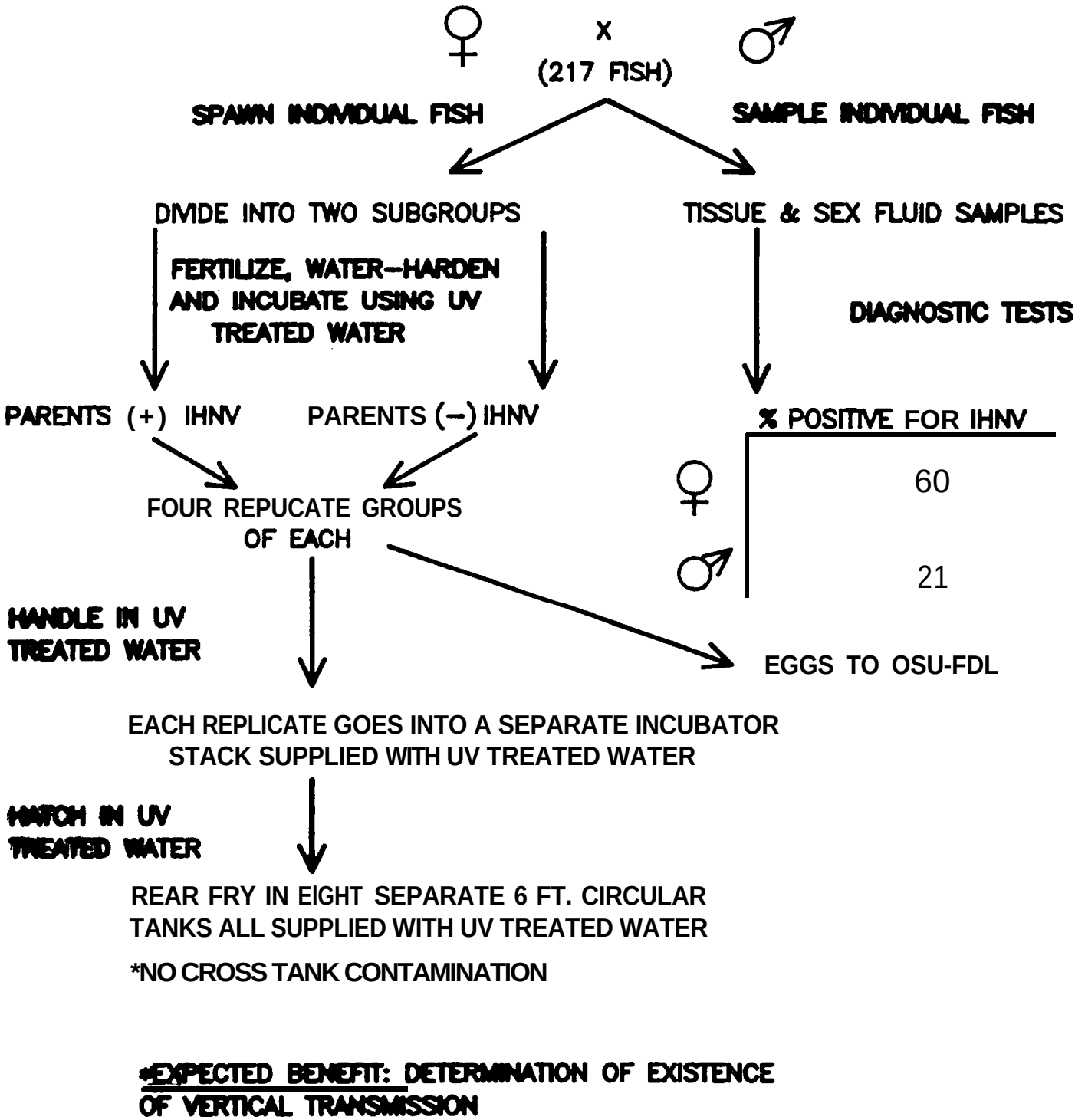
* HORIZONTAL TRANSMISSION = INFECTION VIA THE WATER SUPPLY



• EXPECTED BENEFIT: EFFECTIVENESS OF U.V. STERILIZATION ON REGULAR HATCHERY PRODUCTION AT A KNOWN POSITIVE IHNV FACILITY

"CULL-OUT" CROSSES/VERTICAL TRANSMISSION

*** VERTICAL TRANSMISSION= INFECTION VIA ADULT TO PROGENY**



***EXPECTED BENEFIT: DETERMINATION OF EXISTENCE OF VERTICAL TRANSMISSION**

APPENDIX B

FISH EXAMINATION REPORT

ID No. _____

COLUMBIA RIVER BASIN
FISH EXAMINATION REPORT

LOCATION OF SAMPLE COLLECTION: _____

ORGANIZATION: _____

EXAMINATION DATE(S): _____

SPECIES: _____ STOCK: _____

BROOD YEAR: _____ FISH SIZE(GRAMS): _____

TYPE OF EXAMINATION: ROUTINE _____ DIAGNOSIS _____ PRELIB _____

CERTIFICATION _____ OTHER(PLEASE SPECIFY) _____

_____

MORTALITY: NORMAL _____ INCREASED _____ EPIZOOTIC _____

INCIDENCE OF DISEASE: PERCENT OF INCIDENCE _____

-----OR-----

LOW _____ MODERATE _____ HIGH _____

IF PERCENT OF INCIDENCE: NUMBER OF FISH SAMPLED _____

NUMBER OF DEAD FISH _____

METHOD OF ESTIMATING INCIDENCE _____

_____

REMARKS: _____

_____

_____

_____

_____

_____

PATHOLOGIST(S): _____

DATE EXAMINATION COMPLETED: _____

PATHOGEN EXAMINATION: (please indicate only those pathogens specificallyassayed)

Bacterial Pathogens	Results +	-
Aeromonas sp.		
A. liquifaciens		
A. salmonicida		
Bacterial Gill Disease		
Cytophaga psychrophila		
Edwardsiella tarda		
Flexibacter columnaris		
Mycobacterium sp.		
Renibacterium salmoninarum		
Yersinia ruckeri		
Vibrio anguillarum		
V. ordalii		
Other(please specify)		

Viral Pathogens	Results +	-
IHN Virus		
IPN Virus		
OM Virus(OMV)		
VEN Virus		
VHS virus		
Other (please specify)		

Parasitic and Fungal Pathogens	Results +	-
Ceratomyxa Shasta		
Chloromyxum majori		
C. ward		
Colponema		
Costia		
Cryptobia		
Dermocystidium		
Diplostomulum spathaceum		
Epistylis		
External Fungus		
Gill Amoeba		
Gyrodactylus		
Henneguya		
Hexamita		
Ichthyophthirius		
Internal Fungus		
Myxidium minteri		
Myxobolus insidiosus		
M. kisutchi		
Myxosoma cerebralis		
M. squamalls		
Nanophyetus salmincola		
Neascus		
Piscicola		
PKD		
Plistophora		
Sanguinicola		
Schyphidia		
Trichodina		
Trichophrya		
Other (please specify)		

************OPTIONAL DATA************

WATER TEMPERATURE (°C) _____ DIET _____

LB FEED/GPM _____ FLOW GPM _____ LB FISH/GPM _____

POND TYPE _____ POND VOLUME _____ LB FISH/FT^3 _____

DENSITY INDEX _____ REUSE % _____ REUSE TYPE _____

DATE LAST HANDLED _____ WATER SOURCE _____

DISEASE SIGNS: INTERNAL _____

EXTERNAL _____